MOLECULAR SYSTEMATICS OF THE CHINESE *YINSHANIA* (BRASSICACEAE): EVIDENCE FROM PLASTID AND NUCLEAR ITS DNA SEQUENCE DATA[1]

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ABSTRACT

Species of the Chinese endemic genera *Yinshania*, *Hilliella*, and *Cochleariella* were originally placed in or closely associated with *Cochlearia*. A previous preliminary molecular study mainly on European *Cochlearia* and detailed morphological studies by us showed that this complex was not affined to *Cochlearia* s. str. Depending on the authority consulted, the number of taxa recognized in this complex ranged from 11 to 25 species in one to four genera. The present phylogenetic study is based on the analysis of the ITS (internal transcribed spacer regions of the nuclear ribosomal DNA) and the chloroplast *trn*L-intron sequences from 18 taxa. Resulting phylogenies were compared, and the results demonstrate that there are two different lineages. One lineage combines exclusively the highly polyploid taxa from *Hilliella* and *Cochleariella*. The second lineage includes the diploid taxa from *Yinshania*. However, incongruencies when nrDNA- and cpDNA-derived phylogenies were compared suggest hybridization between these two lineages. We followed a concept to combine all taxa of this complex into the genus *Yinshania*. Our results from phylogenetic analysis of nr and cpDNA support the association of *Yinshania* with *Cardamine* and *Rorippa*, rather than with *Cochlearia*, as was suggested by nearly all previous authors.

Key words: Brassicaceae, *Cochleariella*, *Hilliella*, molecular systematics, reticulate evolution, *Yinshania*.

Many authors follow Schulz (1936) and Schultze-Motel (1986) in dividing *Cochlearia* into the sections *Pseudosempervivum* Boiss., *Glaucocochleria* O. E. Schulz, *Cochlearia* (= *Eucochlearia* Prantl), and *Hilliella* O. E. Schulz. As shown by Koch et al. (1999a), however, this sectional classification is highly artificial. Section *Cochlearia* is widely distributed in Europe and the circumpolar region, whereas section *Glaucocochlearia*, which was raised to the generic rank by Pobedimova (1968), is restricted to southwestern Europe. The latter section consists of *C. glastifolia* L. and *C. megalosperma* (Maire) Vogt, as well as *C. aragonensis* Coste & Soulié, which was only recently included (Koch et al., 1996), although considered to be distantly related to the other two species (Koch et al., 1999a). Section *Cochlearia* consists of a species complex that demonstrates highly polymorphic chromosome numbers, and diverse ecological adaptation and geographic distributions. Morphological differences between phylogenetically sister taxa are often weak and poorly defined (Koch et al., 1996). Both sections *Cochlearia* and *Glaucocochlearia* are closely related to the genus *Ionopsidium* Rchb. (Koch et al., 1999a). Section *Pseudosempervivum*, which is centered in the Middle East and clearly unrelated to *Cochlearia*, is most closely related to *Masmenia* F. K. Mey. and *Noccaea* Moench (Koch et al., 1999a), both of which were segregated by Meyer (1973, 1979, 1991) from *Thlaspi* L. s.l.

The family of Brassicaceae is divided into several tribes and subtribes. Most of them are highly artificial, such as tribe Arabideae (Koch et al., 1999b) or Lepidieae (Zunk et al., 1996). Following classical tribal concepts, *Cochlearia* sect. *Pseudosempervivum*, sect. *Cochlearia*, and sect. *Glaucocochlearia* are members of tribe Lepidieae. Species originally assigned by Schulz (1923) to section *Hilliella* (*Yinshania*, *Hilliella*, and *Cochleariella*) were excluded from *Cochlearia* by Pobedimova (1970), who did not assign them to any genus. However, these species have recently been placed in three Chinese endemic genera, *Yinshania* Y. C. Ma & Y. Z. Zhao, *Cochleariella* Y. H. Zhang & Vogt, and *Hilliella* (O. E. Schulz) Y. H. Zhang, each of which was assigned to a different subtribe. The genus *Yinshania* (Ma & Zhao, 1979) was placed in subtribe

[1] We are grateful to Zhang Yu-hua for providing some of the samples. The curators of A, B, BM, E, GH, HAST, IBSC, K, KUN, LE, MO, NAS, NY, P, PE, TAI, TI, TNS, US, W, and WU are thanked for the loan of specimens.

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Descurainiinae, tribe Sisymbrieae. The genus *Cochleariopsis* (Zhang, 1985), renamed as *Cochleariella* (Zhang & Cai, 1989), was placed in subtribe Cochleariinae, tribe Lepidieae, along with *Hilliella* s. str. (Zhang, 1986). Although a few studies on taxonomy, evolution, and origin of these genera (Zhang, 1987; Zhang & Xu, 1990; Zhang, 1993) have been made, nothing was said about their systematic position in relation to the remaining Asian and European taxa of *Cochlearia*. Zhang's (1987) division of *Yinshania* (excluding *Hilliella*) into two sections and two series and Zhao's (1992) classification of *Yinshania* (including *Hilliella*) into two sections and six series, were shown by Al-Shehbaz et al. (1998) to be highly artificial. In fact, one of the species assigned by Zhang (1987) to *Hilliella* and by Zhao (1992) to *Yinshania* was placed by Al-Shehbaz and Yang (1998) in the synonymy of *Cardamine fragariifolia* O. E. Schulz.

On the basis of a comprehensive morphological survey of *Yinshania*, *Hilliella*, and *Cochleariella*, Al-Shehbaz et al. (1998) reduced the latter two to synonymy of *Yinshania*, and concluded that there is no need for infrageneric subdivisions that do not reflect the phylogenetic relationships of this small genus of 13 species.

They also demonstrated that morphological characters previously used in the delimitation of species (e.g., density of papillae on the fruit valves, fruit shape, and seed number per fruit) are highly variable among and within different populations of the same species. Furthermore, differences in the compression of fruit (terete vs. latiseptate or angustiseptate) that were emphasized heavily by earlier authors (e.g., Schulz, 1936) in the delineation of genera were not found to be taxonomically useful in the *Yinshania* complex. As shown by Koch et al. (1999a), the placement of heavy emphasis on fruit compression has led to the artificial integration of several taxa into *Cochlearia* sect. *Pseudosempervivum* instead of *Thlaspi* s.l. In fact, terete and variously flattened fruits occur in numerous genera of the Brassicaceae, and in many cases this aspect of fruit morphology is taxonomically insignificant.

Morphological convergence and parallelism are widespread in the Brassicaceae (Dvorák, 1971; Meyer, 1973; Avetesian, 1983; Endress, 1992), and the dependence on such characters to construct phylogenies often leads to erroneous conclusions (Sytsma, 1990; Meyer, 1991). Recent molecular analyses (e.g., Warwick et al., 1992; Price et al., 1994; Mummenhoff & Koch, 1994; Zunk et al., 1996; Mummenhoff et al., 1997; Koch et al., 1998a, b; Koch et al., 1999a, b) have made significant contributions to a better understanding of the classification, generic delimitation, and phylogenetic relationships in the Brassicaceae. A preliminary study (Koch et al., 1999a) utilizing ITS nrDNA and cp *trn*L intron sequence data of four species of the *Yinshania* complex (including *Hilliella* and *Cochleariella*) clearly showed that the complex is unrelated to *Cochlearia*. In this analysis it has been shown that ITS and *trn*L intron sequence data provide sufficient sequence variation to distinguish significantly between *Yinshania* and *Cochleariella*/*Hilliella* accessions with both data sets. In order to gain a better insight of the phylogenetic relationships within this Chinese complex, we examined sequence variation of the internal spacer regions (ITS1 and ITS2) of nrDNA (Baldwin et al., 1995; Campbell et al., 1995) and of the cp *trn*L intron (Böhle et al., 1994; Gielly & Taberlet, 1994; van Ham et al., 1994; Koch et al., 1999a), and compared the derived molecular phylogenies with traditional concepts based on morphological data. This approach provided us with the opportunity to characterize species lineages and to analyze incongruencies between different data sets in order to test hypotheses of gene flow over lineages and chloroplast capture.

Materials and Methods

Plant material

Leaf material for DNA extraction was obtained from herbarium specimens (Table 1), most of which were provided and determined by Zhang Yu-hua (Institute of Materia Medica, Zhejiang Academy of Medicine, Hangzhou, People's Republic of China). We did not examine vouchers to verify Zhang's determinations. The samples represent a broad spectrum of species of *Yinshania*, *Hilliella*, and *Cochleariella*. *Cardamine flexuosa* With. and *Rorippa palustris* (L.) Besser served as the outgroups. The DNA sequences for the outgroups were obtained from Franzke et al. (1998).

DNA extraction, PCR-amplification, and sequencing

The total DNA for the outgroups was isolated from leaf tissues following the CTAB (cethyltriammoniumbromide) method of Doyle and Doyle (1987), as modified by Mummenhoff and Koch (1994). DNA extraction from herbarium material was performed in a mini preparation in Eppendorf reaction tubes from 50-μg dried tissue. Tissue was ground with sand and prewarmed 2X CTAB-buffer.

Table 1. Nomenclature, accession data, and GenBank sequence accession numbers for Chinese taxa under study. (Samples 1 through 21 were provided by Zhang, 22 through 26 by Al-Shehbaz.)

Specimen	Nomenclature according to Zhang	Nomenclature according to Al-Shehbaz et al. (1998)	Accession data (China)	Genbank accession code for ITS1 and ITS2 sequences, respectively	Genbank acc. code for the *trn*L intron sequence
1	*Yinshania qianningensis* Y. H. Zhang	Y. *acutangula* subsp. *wilsonii* (O. E. Schulz) Al-Shehbaz et al.	Sichuan, Erdaoqiao, Kangding, *Wu & Cai 9332*	AF100797/AF100798	AF100862
2	*Hilliella yixianensis* Y. H. Zhang	*Y. yixianensis* (Y. H. Zhang) Al-Shehbaz et al.	Anhui, Yixian, *Zhang 95001*	AF100817/AF100818	AF10863
3	*Hilliella sinuata* (Kuan) Y. H. Zhang & H. W. Li var. *qianwuensis* Y. H. Zhang	*Y. sinuata* subsp. *qianwuensis* (Y. H. Zhang) Al-Shehbaz et al.	Jiangxi, Qianwu, *Hu & Li 1761*	AF100833/AF100834	AF100869
4	*Hilliella shuangpaiensis* Y. H. Zhang	*Y. rupicola* subsp. *shuangpaiensis* (Z. Y. Li) Al-Shehbaz et al.	Hunan, Guanjisi, Nanyue, *Wu s.n.*	AF100815/AF100816	AF100865
5	*Yinshania acutangula* (O. E. Schulz) Y. H. Zhang (1)	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	Neimongol, Zaogou, Baotou, Tomoteyouqi, *Zhao Yizhi s.n.*	AF100799/AF100800 AF100801/AF100802	AF100855
6	*Yinshania acutangula* (O. E. Schulz) Y. H. Zhang (2)	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	Xizang, Xinrongxiang, Luolongxian, *Tan 88-3*	AF100803/AF100804	AF100856
7	*Yinshania henryi* (Oliver) Y. H. Zhang	*Y. henryi* (Oliver) Y. H. Zhang	Hubei, Shenlongjia, *Zhou 9107003*	AF100805/AF100806 AF100807/AF100808	AF100859
8	*Yinshania furcatopilosa* (K. C. Kuan) Y. H. Zhang	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang	Hubei, Shenlongjia, *Zhou 9107001*	AF100809/AF100810 AF100811/AF100812	AF100860
9	*Hilliella sinuata* (K. C. Kuan) Y. H. Zhang & H. W. Li	*Y. sinuata* (K. C. Kuan) Al-Shehbaz et al. subsp. *sinuata*	Zhejiang, Sanfeng, Chengan, *Hong 966*	AF100831/AF100832	AF100868
10	*Hilliella alatipes* (Handel-Mazzetti) Y. H. Zhang & H. W. Li var. *micrantha* Y. H. Zhang	*Y. rivulorum* (Dunn.) Al-Shehbaz et al.	Hunan, Jianghua, *collector unknown 7300069*	AF100829/AF100830	AF100878
11	*Hilliella changhuaensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Anhui, Daping, Huangshan, *Wang s.n.*	AF100843/AF100844	AF100864
12	*Hilliella guangdongensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Guangdong, Renhua, *Deng 7298*	AF100813/AF100814	AF100880
13	*Hilliella lichuanensis* Y. H. Zhang	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al.	Jiangxi, Taihe, *Lai 0592*	AF100845/AF100846 AF100847/AF100848	AF100871
14	*Hilliella rupicola* (D. C. Zhang & J. Z. Shao) Y. H. Zhang	*Y. rupicola* (D. C. Zhang & J. Z. Shao) Al-Shehbaz et al.	Anhui, Shitai, *Shao 835008*	AF100823/AF100824	AF100870
15	*Hilliella paradoxa* (Hance) Y. Z. Zhao	*Y. paradoxa* (Hance) Y. Z. Zhao	Sichuan, Beipei, Chongqing, *Wu & Cai 9221*	AF100827/AF100828	AF100866

Table 1. Continued.

Specimen	Nomenclature according to Zhang	Nomenclature according to Al-Shehbaz et al. (1998)	Accession data (China)	Genbank accession code for ITS1 and ITS2 sequences, respectively	Genbank acc. code for the *trn*L intron sequence
16	*Hilliella hunanensis* Y. H. Zhang	*Y. hunanensis* (Y. H. Zhang) Al-Shehbaz et al.	Hunan, Linxian, *Li et al. 646*	AF100825/AF100826	AF100879
17	*Hilliella fumarioides* (Dunn.) Y. H. Zhang & H. W. Li	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Jiangxi, Wulaofeng, Lushan, *Xiong 6729*	AF100819/AF100820 AF100821/AF100822	AF100867
18	*Hilliella warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (1)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Ningbo, *Zheng 5572*	AF100839/AF100840	AF100874
19	*Hilliella warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (2)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Tiantai, *Hong s.n.*	AF100837/AF100838	AF100875
20	*Cochleariella zhejiangensis* (Y. H. Zhang) Y. Z. Zhao (1)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Longquan, *collector unknown 233*	AF100851/AF100852	AF100877
21	*Cochleariella zhejiangensis* (Y. H. Zhang) Y. Z. Zhao (2)	*Y. fumarioides* (Dunn.) Y. Z. Zhao	Zhejiang, Tonglu, *Zhang 97001*	AF100841/AF100842 AF100849/AF100850	AF100876
22	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang	*Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang (A7)	Hubei, Shennog-Jia, *Shennong-Jia Expedition 21828* (PE)		AF100861
23	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula* (A11)	Hebei, Wu-An County, *He 21252* (PE)		AF100857
24	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula*	*Y. acutangula* (O. E. Schulz) Y. H. Zhang subsp. *acutangula* (A12)	Neimongol, Sartchy, *David 2889* (P)		AF100858
25	unknown	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al. (A20)	Jiangxi, Xing Zi County, *Lou 82103* (NAS)		AF100872
26	unknown	*Y. lichuanensis* (Y. H. Zhang) Al-Shehbaz et al. (A21)	Anhui, Huan Shan, Fu Xi, *Shao 810105* (PE)		AF100873

Organic extraction and DNA isolation from herbarium specimens followed Koch et al. (1996).

Double-stranded DNA of the complete ITS region, including the 5.8S rDNA gene, was amplified by 30 cycles of symmetric PCR using ITS primers initially designed by White et al. (1990) and modified by Mummenhoff et al. (1997). The 18F primer (5′-GGAAGGAGAAGTCGTAACAAGG-3′) is located at the 3′-end of the 18S rDNA gene, and primer 25R (5′-TCCTCCGCTTATTGATATGC-3′) is located at the 5′-end of the 25S rDNA. It has been reported that PCR selection of rDNA paralogues has occurred (Buckler et al., 1997). However, PCR selection might have only been important in high G+C content sequences (Buckler et al., 1997). Sequences from *Yinshania* and *Hilliella* (Koch et al., 1999a) are comparable in G+C content to sequences from *Gossypium*, in which PCR selection was probably weak (Wendel et al., 1995a). The resulting amplification product included ITS1, 5.8S rDNA, and ITS2. Only those PCR products were cloned into the pGEM-T-Easy cloning vector (PROMEGA) that showed a single band on ethidium bromide stained agarose gels. Two cloned ITS regions from two independent PCR reactions were sequenced (forward and reverse) with both amplification primers and two universal primers located in the flanking sites of the pGEM-T-Easy vector (t7-forward: 5′-gtaacgatttaggtgacactatcg-3, m13-reverse: 5′-agcggataacaatttcacacagga-3). This means that every single clone was sequenced four times to avoid sequence errors. The *trn*L (UAA) intron was amplified and sequenced by using the universal primer B49318 (5′-CGAAATCGGTAGACGCTACG-3′) located at the 3′-end of the *trn*L(UAA)5′-exon and A49855 (5′-GGGGATAGAGGGACTTGAAC-3′) located at the 5′-end of the *trn*L(UAA)3′-exon (Taberlet et al., 1991). The PCR profile used to amplify the *trn*L intron followed the following profile: hot start with 5 min. at 94°C, and 35 cycles of amplification (1 min. 94°C, 45 min. 50°C, 45 min. 72°C), final elongation step for 10 min. 72°C, and storage at 4°C. DNAs were cycle-sequenced using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (ABI Applied Biosystems, Inc.). Products of the cycle sequencing reactions were run on an ABI 377XL automated sequencer (ABI Applied Biosystems, Inc.). Material from accession numbers 22–26 (Table 1) was only used for sequencing the *trn*L intron sequence, because amplification of the ITS regions failed totally.

DATA ANALYSIS

ITS data. Boundaries of the ITS regions and coding sequences were determined by comparison to those of *Sinapis alba* L. (Rathgeber & Capesius, 1989) and other Brassicaceae (Mummenhoff et al., 1997; Koch et al., 1999a). DNA sequences were aligned by hand. Parsimony analyses were performed with unordered Fitch parsimony and weighted parsimony with a transition:transversion weighting of 1.0:1.08 using PAUP version 3.1 (Swofford, 1993). The BRANCH-AND-BOUND algorithm was used to find maximally parsimonious trees. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates and the HEURISTIC search algorithm with the MULPARS option. We combined the GAPMODE=MISSING option with the coding of the gaps as additional presence/absence characters (Downie & Katz-Downie, 1996). This option decreases the number of equally parsimonious trees because of the redundancy resulting from having two sets of scored characters for the same indel events (Wojciechowski et al., 1993).

Evolutionary data are most often presented as a phylogenetic tree, the underlying assumption being that evolution is a branching process. However, empirical data is rarely ideal and often supports several trees instead of one unique tree. Hence, it makes sense to consider tree reconstruction methods that produce a tree if the given data heavily favor one tree over all others. Otherwise, methods that produce a more general graph that indicates different possible phylogenies are useful (Huson, 1998). One such method is the Split Decomposition introduced by Bandelt and Dress (1992) and its variations. In order to visualize conflicting phylogenetic signals, we analyzed all ingroup ITS sequences (see Fig. 6), using the software program SplitsTree version 1.0.3. (Huson & Wetzel, 1995 shareware ftp://ftp.uni-bielefeld.de/pub/math/splits/).

trn*L intron data.* DNA sequences were aligned by hand. Parsimony analysis was performed with PAUP (version 3.1; for options, see ITS data). Gaps were treated as additional unweighted binary characters. These gaps were coded using strict criteria: gaps must occur at the same position and have the same aligned length to be treated as homologous, and no splitting of one gap in two or more characters was performed (Koch et al., 1999a). Bootstrap analysis was performed as described above.

TRIBAL RELATIONSHIPS

To estimate the tribal affinity of *Yinshania*, we derived an ITS phylogeny with sequences from *Capsella rubella* Reut. (Koch et al., 1999b), *Arabidopsis thaliana* (L.) Heynh. (GenBank U43224), *Yinshania acutangula* (O. E. Schulz) Y. H. Zhang,

Barbarea vulgaris R. Br. (EMBO X98632), *Cardamine flexuosa* With. (Franzke et al., 1998), *Thlaspi arvense* L. (Koch et al., 1999a), *Cochlearia aestuaria* (Lloyd) Heywood (Koch et al., 1999a), *Brassica oleracea* L. (GenBank AF039994/AF040038), and *Sinapis alba* (EMBO X66325). DNA sequences were aligned by hand, and the alignment is shown in Figure 4. Alignment positions 109–160 were removed from subsequent analysis. Parsimony analyses were performed with unordered Fitch parsimony using PAUP version 3.1 (Swofford, 1993). The BRANCH-AND-BOUND algorithm was used to find maximally parsimonious trees with the GAPMODE=NEWSTATE option. Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates and the HEURISTIC search algorithm employed with the MULPARS option. A decay analysis (Bremer, 1988) was performed in addition to the bootstrap approach, in order to assess the confidence that could be placed in the monophyly of clades. Decay indices (DI) were estimated according to Baum et al. (1994).

Results

ITS data

The total length of the alignment with accession numbers 1–21 and outgroups *Cardamine flexuosa* and *Rorippa palustris* is 464 bp, with 283 and 181 nucleotides in ITS1 and ITS2 spacer regions, respectively. The alignment required 36 (7.8%) gap positions, including the outgroups, and is shown in Figure 1. Sequence data were submitted to GenBank with accession numbers AF100793–AF100852 (Table 1). One third of these gaps is located between positions 117 and 133 in the ITS1. This region was completely excluded from subsequent analysis. Additionally, we excluded nucleotide position 465–467 and 473–484 from subsequent data analysis because of an ambiguous alignment (Fig. 1). Gaps from nucleotide positions 11–14 and 293–294 were treated as one single gap, respectively. The number of introduced gaps is comparable to a phylogenetic analysis of *Thlaspi* s.l. with 4.8% of the sites (Mummenhoff et al., 1997). In *Krigia* and outgroups, Kim and Jansen (1994) had to introduce gaps in 3.9% of the sites. Total lengths of ITS1 and ITS2 are nearly identical among the taxa surveyed, and vary between 447 bp (*Hilliella alatipes* (Hand.-Mazz.) Y. H. Zhang & H. W. Li var. *micrantha* Y. H. Zhang [= *Yinshania rivulorum* (Dunn) Al-Shehbaz et al.], accession no. 10) and 457 bp (*Cardamine flexuosa* and *Rorippa palustris*).

Phylogenetic analysis using Fitch parsimony, including the additional 0/1 matrix for the gap position, resulted in 24 most parsimonious trees (MPTs) with a length of 538 and a consistency index (CI) of 71.4% (66.9% if autapomorphies are excluded). Of the 264 variable nucleotide positions, 166 informative positions were in the ITS1 region (including 48 autapomorphies) and 98 in the ITS2 region (including 25 autapomorphies). Four out of 36 gap positions within the total sequence alignment are unique to a particular sequence (*Rorippa palustris* 2 gaps, acc. no. 3, and acc. no. 14). A calculation of the transition/transversion ratio for the MPTs revealed a ratio of 1.00:1.08. Therefore, we used a weighted parsimony approach with a character state weighting of 1.00:1.08 (transition:transversion), which resulted in one MPT that is also represented among the 24 MPTs from the Fitch parsimony with a consistency index of 67.7% (CI 57.9% if autapomorphies were excluded). We present the MPT from the weighted parsimony approach to demonstrate relative branch length (Fig. 2). Bootstrap values are provided from 1000 replicates using the weighted parsimony approach. For most taxa we identified only one ITS sequence type within a single specimen. For *Hilliella lichuanensis* Y. H. Zhang (acc. no. 13), *Cochleariella zhejiangensis* (Y. H. Zhang) Y. H. Zhang & R. Vogt (acc. no. 21), *Yinshania acutangula* (acc. no. 5), *Y. henryi* (Oliv.) Y. H. Zhang (acc. no. 7), and *Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang (acc. no. 8), we found two very similar ITS sequences among the two clones sequenced. In the case of accession numbers 13, 5, 7, and 8, ITS1 and ITS2 regions differed by only a single site mutation. In the case of *C. zhejiangensis* (acc. no. 21), the two ITS types from the same individual differed by 15 mutations, which might indicate that two different ITS loci were cloned and sequenced. Both sequences clustered within the same clade. In *Hilliella fumarioides* (Dunn) Y. H. Zhang & H. W. Li (acc. no. 17), we detected two ITS sequences from a single individual that clustered in different clades (Figs. 2, 6). Both sequences differed by 76 mutations. The ITS data clearly support a separation of two clades consisting of *Yinshania* sensu Zhang (1987) and *Hilliella/Cochleariella.* Different accessions from one taxon (sensu Al-Shehbaz et al., 1998, refer to fig. 2) grouped at different positions in the case of *C. zhejiangensis* (acc. no. 20, not related to acc. nos. 18, 19, 21).

Using the taxonomic concept of Al-Shehbaz et al. (1998) and merging *Hilliella changhuaensis* Y. H. Zhang, *H. guangdongensis* Y. H. Zhang, and *H. lichuanensis* (acc. nos. 11, 12, 13, respectively) into *H. lichuanensis*, only accession numbers 11 and 13

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GAPS                    ****   *                                 *
          1                                                              60

C. FLEXUOSA   TCGTATCCTGCCCAAAA-AAGACCGAACCGCGGACCAAAGATCATCAACTCTGGTGAGCG
R. PALUSTRIS  TCGATCCTGTACATAAA-CAGAACGACCCGCGAACCAAAGATCATCACTCACGGTGGGCC
1             TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
2             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
3             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
4             TCGATACCTGTCCCGAA-CAGAACGACCCGTGAACAATCGATCACCACTCTCGGTAGGCC
5a            TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
5b            TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
6             TCGATACCTGTCCAAAACCAGAACGACCCGCGAACCAAAGATCATCACTCGCG-TAGGCC
7a            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
7b            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTAGGCC
8a            TCGATACCTGTCCAAAAACAGAACGACCTGCGAACCAAAGATCATCACTCGCGGTCGGCC
8b            TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAAAGATCATCACTCGCGGTCGGCC
9             TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
10            TTGTCTTAACCTGGAAA-CAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
11            TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
12            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
13a           TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
13b           TCGATACCTGTCCCGAA-CAGAACGACCTGCGAACAACCGATCATCACTCTCGGTAGGCC
14            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
15            TCGATACCTGTCCCGAA-CGGAACGACCCGCGAACAACCGATCATCACTCTCGGTAGGCC
16            TCGTAACCTG----GAAACAGAACGACCCGAGAACGTTGAAACATCACTCTCGGTGGGCC
17a           TCGATACCTGTCCCGAA-CAGAACGACCCGCGAACAATCGATCACCACTCTCGGTAGGCC
17b           TCGATACCTGTCAAAAA-CAGAACGACCCGAGAACGATTAATCATCACTCTCGGTAGGCC
18            TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
19            TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
20            TCGATACCTGTCCAAAA-CAGAACGACCCGCGAACCGAAGATCATCACTCGCGGTAGGCC
21a           TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
21b           TCGATACCTGTCCAAAA-CAGAACGACCAGCGAACTTTCGATCAACACTCCCGGTAGGCC
```

Figure 1. Alignment of ITS1 and ITS2 regions from *Yinshania*, *Cochlearia*, and *Hilliella*, as well as outgroups *Cardamine flexuosa* and *Rorippa palustris*. Characters excluded from data analysis are marked (Δ). Gap positions 1–4 were treated as a single binary 0/1 character. Taxon enumeration follows Table 1.

```
                    *                   *                 *    ********** *   *
   61                                                      [del. Δ: 117 - 133]                                                  160
                                                           ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ
   GGTTTCATAACTTGAGATCGTGCCTGCCGAATCCGTGGTTTCGCCGAACAATCCTTACCGGGAGCTTAATCTCTGTTTGGATTGTGCGCGTTGCTTCCGG
   GGTTTCTTAGCCGAGATC-GTGCCTGCCGAATCCGTGGTTTCGCGAGCAATCCTTACCGGGAGCTCT-ATCTCGGTTTGGGTTGTGCGCGTTGCTTCCGG
1  GGTTTCTTAGCCGATTCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGGTAGTTCCGG
2  GGATTCCCGACGGTCTCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---TCTTGCCTCGGC-GGGTTCGTGCGCGCAGCTTCCGG
3  GGTTCTTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGCGTATGTCTCCATCGAGA--GTTTTACCTCGGT-GGGTTCATGCACGCGGCTTCCGG
4  GGTTCCTAAACAGTCCTC-GCGTCTACCAATTCCGTGGTTT-GAGCACGTCTACATGAGAG---TTTTACCTCGGT-GGATTCATGCACGCGGCTTCCGG
5a GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
5b GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
6  GGTTTCTTAGCCGATCCCGGTGCCTGCCGTGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCT-ATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
7a GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-AATCTCGGT-CGTGTCGTGCGCGTAGCTTCTGG
7b GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-AATCTCGGT-CGTGTCGTGCGCGTAGCTTCTGG
8a GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-TATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
8b GGTTTCTTAGCCGATCCC-GTGCCTGCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTC-TATCTCGGT-CGTGTCGTGCGCGTAGCTTCCGG
9  GGTTCCTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGCGTATGTCTCCATCGAGA--GTTTTACCTCGGT-GGGTTCATGCACGCGGCTTCCGG
10 GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
11 GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCCCGGC-TGGTTCGTGCGCGCAGCTTCCGG
12 GGTATCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-CGGATTGTACGCATAGCTTCCGG
13a GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
13b GGGTTCCAGACGGTCCCC-GCGCCTACCGATTCCGTGGTTTCGCGCACGTTTCAGCCAAGG---CCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
14 GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTTGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
15 GGGTTCCCGACGGTCCCC-GCGCCTACCGATTCCGTGGTTCCGCGCACGTTTCAGCCAAGG---TCTCGCCTCGGC-TGGTTCGTGCGCGCAGCTTCCGG
16 GGTTTCTTAGCTGATTTC-GTGCCTACCGATTCCGTGGTTATGCGTTCGTCACCGGCCCAG-----TT---TCGGT-TGGATCATACGCATAGCTTCCGG
17a GGTTCCTAAACAGTCCTC-GCGTCTACCGATTCCGTGGCTTCGAGCACGTCTCCATCAGAG---TTTTACCTCGTT-GGGTTCATGCACGCGGCTTCCGG
17b GGTTTCTTAGCTGATCCC-GTGCTTGCCGATTCCGTGGTTTCGCGTATCGTCCCTTCAAGA---TTTTATCTCGGC-TGGGTCATGCGTGTTGCTTCCGG
18 GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCACGTTTCCCT-TAAAATGTTTTACATTGGT-TGGATCATGGGCGCGGCTTCCGG
19 GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGGCGCGGCTTCCGG
20 GGTTTCTTAGCCGATCCC-GTGCCTACCGATTCCGTGGTTCATGTTTGGCTTCGTC-AGTA-TTCATTACAT-GGC-GAAGTCATGCATGTTTCCTCCGG
21a GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGGCGCGGCTTCCGG
21b GGTTTCTTGACAGATCTC-GTGCCTACCGGATCCGTGGTCTCGCTCATGTTTCCCTCAAAAATGTTTTACATTGGT-TGGATCATGGGCGCGGCTTCCGG
```

Figure 1. Continued.

```
                 *                                                                                          *
    161                                                                                                   260

    ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCAATTGAACAGACAGCCTTCGCCTCCCCGGAGACGGTGTGTGTGCGGATCCTGCGCTGCG
    ATATCACAAAACCCCGGTACGAAAAGTGTCAAGGAACATGCAATTGAACAGCCAGCCTTCGCCTCCCCGGTCACGGTGCGTGTGCGGTTNCTGTNCTNCG
1   ATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCACGGTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTACG
2   ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGCACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
3   ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCGGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGTTGCCGG-CTGCA
4   ATATCACAAAACCACGGCACGAAAAATGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGAAGCCGTGCTGCA
5a  ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
5b  ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
6   ATATCACCAAACAC-GGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
7a  ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
7b  ATATCCCCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGCG
8a  ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGAAACGGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGTG
8b  ATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGAAACAGAACGGCCAGCCTTCGCCTCCCCGGATACGGTGTGAGTGCGGATGTTGTGCTGTG
9   ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCGGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCCGTGCTGCA
10  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAAACGGTGTTTGTTCGGAAACAGTGTTGCA
11  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGNNNNNNN
12  ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACAGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
13a ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
13b ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATTGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
14  ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
15  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGATGCAGCGCCGCG
16  ATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTTGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
17a ATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATCGAACCGATCGGCCTGCCTTCGCCGCCCCGGAGACGGTGCGCGTGCGGAAGCCATGCTGCA
17b ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCTTGCGTTCGCCTACCCGGAGACGGAGACGGTGTGGTGCGGATGCTGCA
18  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
19  ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
20  ATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATTCAACTAAACGGCCTGCTTTCGCCAACCCGGAGACGGTGTTTGTTCGGAAGCAGTGCTGCA
21a ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGTCTTCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
21b ATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCTGCCTCCGCCGCCCCGGAGACGGTGTGCGCGCGGATGCAGTGCTGCT
```

Figure 1. Continued.

```
                          *     **          *                  *
     261                  283ITS2                                                           360

     ATCTAAAGTCTATCGTCGTCCCTCTCATCCTTCTTAGGACGTGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCCAAATCCGAGCTA
     ATCTAAAGTCTATCGTCGTCCCTCTCATCCTTC-TCGGATATGGGACGGAAGCTGGTCTCCCTTGTGTTA-CCGCATGCGGTTGGCCGAAATCCGATCTA
1    ATCTAAAGTCTATCGTCGTCCATTTCATTCCTATAAGGATCCGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
2    ATCTAAAGTCTACCGTCGTCCCCTACATCCCCGAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
3    ATCTAAAGTCTACCGTCGTCCCC-ACATCCCCTAAGGGTACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
4    ATCTAAAGTCTACCGTCGTCCTCTACATCCCCCAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
5a   ATCTAAAGTTTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
5b   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
6    ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
7a   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTT--GCGGATACGGGACGGA-GCTGGTCTCCCGTGTGTTA-CCGCACGCCGTCGGGCAAAATCCGAGCAA
7b   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTT--GCGGATACGGGACGGA-GCTGGTCTCCCGTGTGTTA-CCGCACGCCGTCGGGCAAAATCCGAGCAA
8a   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
8b   ATCTAAAGTCTATCGTCGTCCCCTTCATCCTTT-GCGGATACGGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCAA
9    ATCTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCGAGCTA
10   AGGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGCGTTA-CCGCACGCGGTTGGCCAAAATCCTAGCTA
11   NNNNAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
12   ATGTAAAGTCTATCGTCGTCCCC-CAATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGNACGCGGTTGGCCAAAATCCGAGCTA
13a  ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
13b  ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
14   ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGCCATCCTCTCGAGGATGGCCAAAATCCTAGCTA
15   ATCTAAAGTCTACCGTCGTCCCC-ACATCCCC-CGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCGAAATCCGAGCCA
16   ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGACGCCGGTTGGCCAAAATCCTAGCTA
17a  ATCTAAAGTCTACCGTCGTCCCC-ACATCCCCTAAGGATACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCGAAATCCGAGCTA
17b  ATCTAAAGTCTACCGTCGTCCCC-NNATCCTTTCGAGGATGCTGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCATGCGGTTGGCCAAAATCCGAGCAA
18   AAATAAAGTCTATCGTCGTCCCC-ACATCCCTTCGAGGACACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTTGCCAAAATCCGAGCTA
19   AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCTCGCGGTTTGCCAAAATCCGAGCTA
20   ATGTAAAGTCTATCGTCGTCCCC-CCATCCTCTCGAGGATATCGGACGGAAGCTGGTCTCCCGTGTGTTA-CCGCACGCGGTTGGCCAAAATCCTAGCTA
21a  AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCGGTGTGTTA-CCGCTCGCGGTTTGCCAAAATCCGAGCTA
21b  AAATAAAGTCTATCGTCGTCCCC-ACATCCCCTCGAGGACACGGGACGGAAGCTGGTCTCCCGTGTGGTA-CCGCACGCGGTTGGCCAAAATCCGAGCTA
```

Figure 1. Continued.

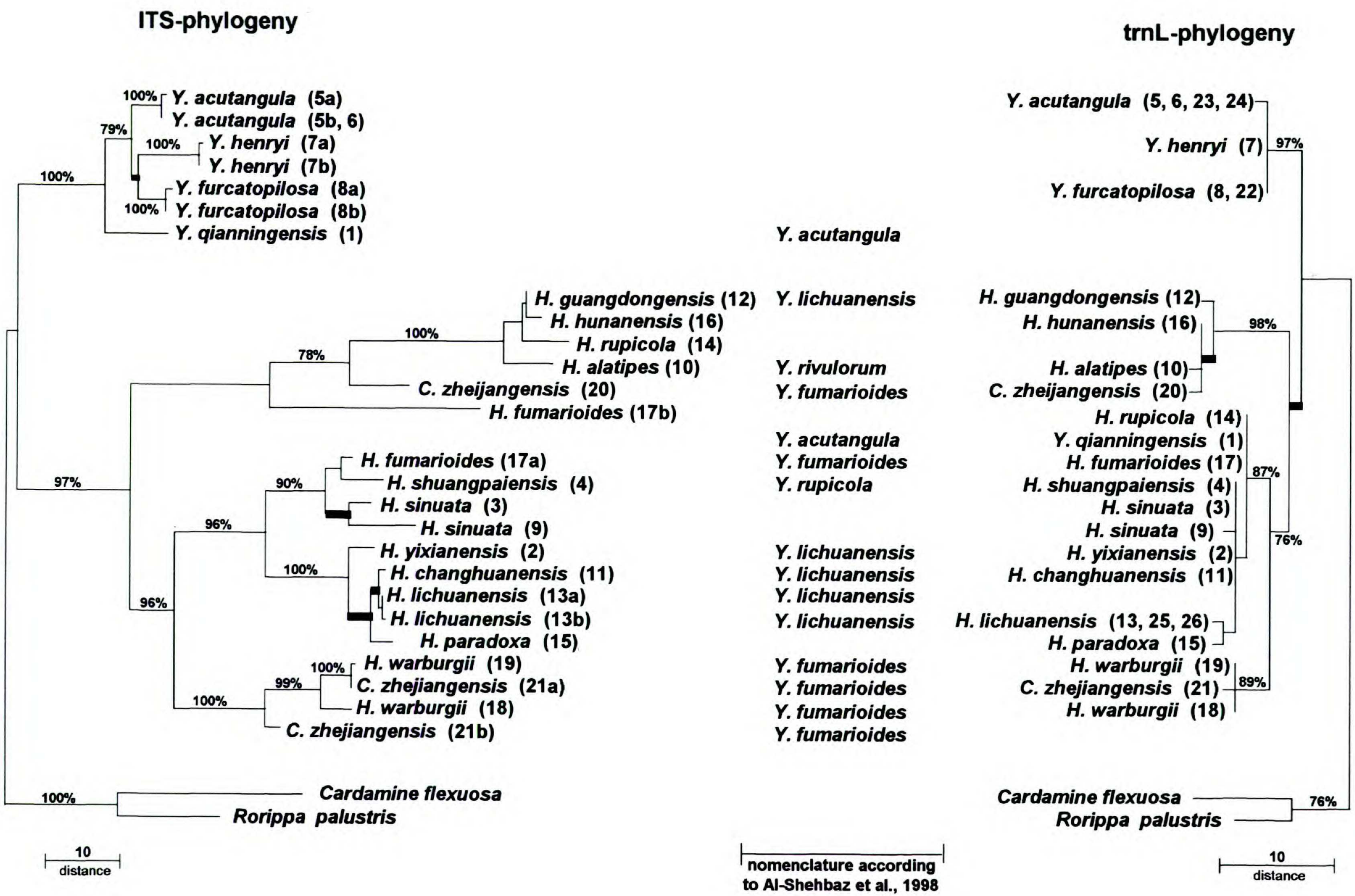
```
        *     *                                     *     **                    *        *  *
     361                                                                                              460

     AGG-ACGCCGAGAGCGTCACGACATGCGGTGGTGAACTAAAGCCTGT-TCGTA--TCGTCGGTCGTTCTTGTCTATAAGCTCTCGATGACCCAAAATCCT
     AGG-ACGCCGAGAGCGTCACGACATGCGGTGTTGAACTAAAGCCTCT-TCGTCAGTCGTCGGTCGTTCTTGTCCGGAAGCTCTCGATGACCCAAAGTCCT
1    AGG-ACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCGATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
2    AGG-ACGTAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTTCTGTCCGGACGCTCTAGTTGACCCAAAGTCCT
3    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCAAAGTTCT
4    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATCACCCAAAGTTCT
5a   AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
5b   AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
6    AAGGACGCC-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGTCGCTCCTGTCCGAAAGCTCTAGATGACCCAAAGTCTT
7a   AAGGACCCG-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATAATA--TCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGT-TT
7b   AAGGACCCG-GGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATAATA--TCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGT-TT
8a   AAGGACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTTGATGACCCAAAGTCTT
8b   AAGGACGCCGGGAGCGTCCCGACATGCGGTGGTGTGTGCAAGCCTCTATCATA--TCGTCGGCCGCTCCTGTCCGAAAGCTCTTGATGACCCAAAGTCTT
9    AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CCGTCGGGCGTTTCTGTCCGGATGCTTTTTATGACCCAAAGTTTT
10   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
11   AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGACGCTGTAGACGACCCAAAGTCCT
12   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCANAGTCCT
13a  AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTAGACGACCCAAAGTCCT
13b  AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTAGACGACCCAAAGTCCT
14   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCCATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCTTTGTCCT
15   AGG-ACGCAGGGAGCGCCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGACGCTCTAGACGACCCAAAGTCCT
16   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
17a  AGG-GCGCCAGGAACGTCCCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--CGGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCAAAGTTCT
17b  AGG-ACGCCTGGCGCGTCTCGACATGCGGTGGTGGATTCAATTCTCG-TCAAA--TTGTCGGTCGTTTCTGTCCGGAAGCTCTCGATGACCCAAAGTCCT
18   AGG-ACGCTAGGAGCCGTCTCGCATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCGCTCCTGTCCGGATGCTCTCGATGACCCGATTCGTT
19   AGG-ACGCTGGGAGCCGTCTGGCATGCGGTGGTGAATCCAAGCCTTG-TCATA--TCGTCNNCCCGCTCTGTTCCGATGCTTTNGATGACCCGATTCGTT
20   AGG-ATGCCAGGAGCGTCTTGACATGCGGTGGTGAATTCAATTCTCG-TCAAA--TCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCT
21a  AGG-ACGCTGGGAGCCGTCTGGCATGCGGTGGTGAATCCAAGCCTTG-TCATA--TCGTCNNCCCGCTCTGTTCCGATGCTTTNGATGACCCGATTCGTT
21b  AGG-ACGCTAGGAGCGTCTCGACATGCGGTGGTGAATCCAAGCCTCG-TCATA--TCGTCGGCCCGCTCTGTCCGGACGCTCTAGACGACCCAAAGTCCT
```

Figure 1. Continued.

		gaps: ITS1	ITS2
	466		
	CAAA	11110111111	1111001000111
	CAAA	11110101111	1101001011111
1	CAAA	11111101011	1111001100111
2	CAAC	11110101011	1111001000111
3	CAAC	11110101010	0111001000111
4	CAAC	11110100011	1111001000111
5a	CAAA	11111011001	1101010100111
5b	CAAA	11111011001	1101010100111
6	CAAA	11111011001	1101010100111
7a	CAAG	11111101011	1000110100000
7b	CAAG	11111101011	1000010100000
8a	TCAA	11111101011	1101011100111
8b	TCAA	11111101011	1101011100111
9	CAAC	11110101011	0111001000111
10	CAAC	11110101011	0111001000111
11	CAAC	11110101011	0011001000111
12	CAAC	00001101011	0111001000111
13a	CAAC	11110101011	0011001000111
13b	CAAC	11110101011	0011001000111
14	CAAC	00001101011	0111101000111
15	CAAC	11110101011	0011001000111
16	CAAC	00001101011	0111001000111
17a	CAAC	11110101011	0111001000111
17b	CAAC	11110101011	0111001000111
18	CAAC	11110101011	0111001000111
19	CAAC	11110101011	0111001000111
20	CAAC	11110101011	0111001000111
21a	CAAC	11110101011	0111001000111
21b	CAAC	11110101011	0111001000111

Figure 1. Continued.


ITS-phylogeny
trnL-phylogeny
Y. acutangula (5a)
Y. acutangula (5b, 6)
Y. henryi (7a)
Y. henryi (7b)
Y. furcatopilosa (8a)
Y. furcatopilosa (8b)
Y. qianningensis (1)
H. guangdongensis (12)
H. hunanensis (16)
H. rupicola (14)
H. alatipes (10)
C. zheijangensis (20)
H. fumarioides (17b)
H. fumarioides (17a)
H. shuangpaiensis (4)
H. sinuata (3)
H. sinuata (9)
H. yixianensis (2)
H. changhuanensis (11)
H. lichuanensis (13a)
H. lichuanensis (13b)
H. paradoxa (15)
H. warburgii (19)
C. zhejiangensis (21a)
H. warburgii (18)
C. zhejiangensis (21b)
Cardamine flexuosa
Rorippa palustris
100%
79%
100%
100%
100%
78%
97%
90%
96%
100%
96%
100%
99%
100%
10
distance
Y. acutangula
Y. lichuanensis
Y. rivulorum
Y. fumarioides
Y. acutangula
Y. fumarioides
Y. rupicola
Y. lichuanensis
Y. lichuanensis
Y. lichuanensis
Y. lichuanensis
Y. fumarioides
Y. fumarioides
Y. fumarioides
Y. fumarioides
nomenclature according
to Al-Shehbaz et al., 1998
Y. acutangula (5, 6, 23, 24)
Y. henryi (7)
Y. furcatopilosa (8, 22)
H. guangdongensis (12)
H. hunanensis (16)
H. alatipes (10)
C. zheijangensis (20)
H. rupicola (14)
Y. qianningensis (1)
H. fumarioides (17)
H. shuangpaiensis (4)
H. sinuata (3)
H. sinuata (9)
H. yixianensis (2)
H. changhuanensis (11)
H. lichuanensis (13, 25, 26)
H. paradoxa (15)
H. warburgii (19)
C. zhejiangensis (21)
H. warburgii (18)
Cardamine flexuosa
Rorippa palustris
97%
98%
87%
76%
89%
76%
10
distance

grouped together by ITS data. Accession number 12 (*H. guangdongensis*) is unrelated to these accessions based on ITS data. Similarly, *H. warburgii* (O. E. Schulz) Y. H. Zhang & H. W. Li (acc. nos. 18, 19), *Cochleariella zhejiangensis* (acc. nos. 20, 21), and *H. fumarioides* (acc. no. 17) merge into *Y. fumarioides* (Dunn) Y. Z. Zhao, as proposed by Al-Shehbaz et al. (1998). ITS sequences from *Y. fumarioides* sensu Al-Shehbaz et al. (1998) are found in three different positions among the *Hilliella/Cochleariella* clade (Fig. 2).

*trn*L DATA

The alignment of 514 bp is interspersed with seven gaps as shown in Figure 3. Sequence data were submitted to GenBank with accession numbers AF100853 through AF100881 (Table 1). The lengths of *trn*L intron sequences range from 311 bp in *Hilliella rivulorum* to 514 bp in *Cardamine flexuosa*. Of the 38 variable nucleotide positions in the alignment, 12 sites are autapomorphic. In addition, one of the seven gaps is autapomorphic. Phylogenetic analysis resulted in two MPTs with a length of 53 steps and a consistency index of 92.5% (90.0% if autapomorphies were excluded). One most parsimonious tree is shown to demonstrate relative branch length. The strict consensus tree could be generated easily by drawing branches that are indicated in Figure 2 by heavy bars with zero length. Bootstrap values are given from 1000 replicates.

Discrimination of a *Yinshania* clade from a *Cochleariella/Hilliella* clade is not as obvious with *trn*L intron data as compared to ITS sequence data (Fig. 2). In the *trn*L tree the branch setting of the *Yinshania* clade as a sister group to the *Hilliella/Cochleariella* clade is not highly supported, and bootstrap value for this branching point is less than 50% (Fig. 2). Removing additional gap characters from the matrix parsimony analysis resulted in one MPT with the *Yinshania* clade as a sister group to *H. hunanensis* (acc. no. 16), *H. guandongensis* (acc. no. 12), *C. zhejiangensis* (acc. no. 20), and *H. alatipes* var. *micrantha* (acc. no. 10). Remaining *Hilliella/Cochleariella* taxa appeared in this analysis for *trn*L data excluding gap information as a sister group to these two clades. Nonetheless, integration of *Yinshania qianningensis* Y. H. Zhang into the *Hilliella/Cochleariella* clade is significant for *trn*L data, in contrast to its segregation by ITS. Estimation of decay indices (DI) using the *trn*L intron matrix without gap information revealed a high value, DI = 3+, for the branch setting of the *Yinshania* clade. Different accessions of *C. zhejiangensis* (acc. nos. 20, 21) did not group closely together; this has also been documented for the ITS data (Figs. 2, 6). *Hilliella changhuaensis*, *H. guangdongensis*, and *H. lichuanensis* do not group together in the cpDNA-based tree as proposed by the morphology-based concept combining them in *H. lichuanensis* (Al-Shehbaz et al., 1998); the same discordance holds for *H. warburgii*, *C. zhejiangensis*, and *H. fumarioides*. Based on the *trn*L sequence data, they are not combined in one single clade that could be named as *H. fumarioides* as proposed in the revison of Al-Shehbaz et al. (1998).

ITS VERSUS TRNL INTRON DATA

Both phylogenetic trees (ITS vs. *trn*L data) from Figure 2 show some congruencies (all following arguments are also true when comparing the strict consensus trees from Fitch parsimony, which could be easily deduced by drawing branches indicated by heavy bars with zero length):

(1) *Hilliella guangdongensis* [sensu Zhang], which has been merged in *H. lichuanensis* sensu Al-Shehbaz et al. (1998), is separated from remaining *H. lichuanensis* sensu Al-Shehbaz et al. (1998), and it is more closely related to *H. hunanensis*, a taxon that was recognized by Al-Shehbaz et al. (1998).

(2) *Hilliella warburgii* and *Cochleariella zhejiangensis* are not integrated into *H. fumarioides* sensu Al-Shehbaz et al. (1998), but (3) most accessions

←

Figure 2. Comparison of the ITS-derived phylogeny with those from the *trn*L sequence data from *Yinshania*, *Cochleariella*, and *Hilliella*, as well as outgroups *Cardamine* and *Rorippa* from subtribe Arabideae (Brassicaceae). Accession enumeration in brackets follows Table 1. For the ITS and the *trn*L tree the nomenclature sensu Zhang (1985, 1986, 1987, 1993) has been used. In between both trees the taxonomic treatment of these taxa according to Al-Shehbaz et al. (1998) is shown. The most parsimonious tree (weighted parsimony approach) from ITS nuclear sequence data and one out of two MPTs from the *trn*L plastid sequence data is shown to demonstrate relative branch length. Weakly supported branches that collapsed in the strict consensus trees using Fitch parsimony are highlighted by a broad line. (The strict consensus trees could therefore be easily generated by drawing broad lines as zero branches.) Bootstrap values are provided from 1000 replicates and shown above branches if greater than 50%. Distance scale indicating branch lengths is given below. ITS designation (a) and (b) of accession nos. 5, 7, 8, 13, 17, and 21 indicate different ITS types from a single individual.

```
      gaps:          a     b
             1                                                                                              100

C. FLEXUOSA  AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTTGT
R. PALUSTRIS AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTTGT
5-6-24-25    AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG
7-8-23       AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG
1-14-17      AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
2-3-4-11     AATTGGAT-GAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
15           AATTGGAT-GACCC-TGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
9            AATTGGAT-GAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
13-26-27     AATTGGAT-GAGCC-TGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
18-19        AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
21           AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCCGGT
20           AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG
10           AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG
16           AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG
12           AATTGGATTGAGCCTTGGTATGGAAACCTACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGCAATCCTGAGCCAAATCCTGGG


      gaps:                                 c
             101                                                                                            200

C. FLEXUOSA  TTACGCGAACAAACCTGAGTTTAGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGAAGTTCACTACCTTGTGTTG
R. PALUSTRIS TTACGCAAACAAACCCGAGTTTAGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
5,6,24,25    TTACGCGAACAAACCGGAGTTTCGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
7,8,23       TTACGCGAACAAACCGGAGTTTCGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
1,14,17      TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
2,3,4,11     TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
15           TTACGCGAACAAACCAGAGTTTAGAAGGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
9            TTACGCGAACAAACCAGAGTTTAGAAGGTGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
13,26,27     TTACGCGAACAAACCGGAGTTTAGAAGGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACTTTGTGTTG
18,19        TTACGCGAACAAACCAGAGTTTAGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
21           TTACGCGAACAAACCGGAGTTTAGAAAGCGAGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCACTACCTTGTGTTG
20           TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
10           TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
16           TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
12           TTACGCGAACAAAACAGAGTTTAGAAAGC---------GGGATAGGTGCAGAGACTCAATGGACGCTGTTCTAACAAATGGAGTTCAATCCCTTGTGTTG
```

Figure 3. Alignment of the plastid *trn*L intron region from *Yinshania*, *Cochlearia*, and *Hilliella*, as well as outgroups *Cardamine flexuosa* and *Rorippa palustris*. Taxon enumeration follows Table 1. Gaps are marked alphabetically, and the corresponding gap matrix is added.

```
        gaps: d                                          e        f
              201                                                                                       300

C. FLEXUOSA   -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGCAGGAGAAAAGCCTATATTGTCTAAATAAAGGTAACACAAAACGATCTCAAAAACGA
R. PALUSTRIS  -ATAAAGGAATCCTTCGATCGAAACTTCAACTCAAAAAGGATGAAGGAGAAA----------GTCTGAATATAGGTAACACAAAACGATCTCAAAAATAA
5,6,24,25     -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGAATGAGAAAAACCTATATTGTCTAAATATAGGTAACACAAAACGATCTCAAAAATGA
7,8,23        -ATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAGGATGAATGAGAAAAACCTATATTGTCTAAATATAGGTAACACAAAACGATCTCAAAAATGA
1,14,17       -AT-------------------------------------------------------------------------------------------------
2,3,4,11      -AT-------------------------------------------------------------------------------------------------
15            -AT-------------------------------------------------------------------------------------------------
9             -AT-------------------------------------------------------------------------------------------------
13,26,27      -AT-------------------------------------------------------------------------------------------------
18,19         -AT-------------------------------------------------------------------------------------------------
21            -AT-------------------------------------------------------------------------------------------------
20            AAT-------------------------------------------------------------------------------------------------
10            AAT-------------------------------------------------------------------------------------------------
16            AAT-------------------------------------------------------------------------------------------------
12            AAT-------------------------------------------------------------------------------------------------


        gaps:
              301                                                                                       400

C. FLEXUOSA   CGACCTGAATCTCGATTTCTATTTTTTTATAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGACAAAATCAAATATTCATTGATCAAATAAT
R. PALUSTRIS  CGACCTGAATCTCGATTTCTATTTTTTTATAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAGAAAATCAAATATTCATTGATCAAATGAT
5,6,24,25     CGACCTGACTCTCGATTTCTATTTTTTTCTAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAAAAAAATCGAATATTCATTGATCAAATGAT
7,8,23        CGACCTGACTCTCGATTTCTATTTTTTTCTAAACAAAATAGAAATGTTGTGAATCAATTCGAAGTTTAAGAAAAAAATCGAATATTCATTGATCAAATGAT
1,14,17       ----------------------------------------------------------------------------------------------CAAATGAT
2,3,4,11      ----------------------------------------------------------------------------------------------CAAATGAT
15            ----------------------------------------------------------------------------------------------CAAATGAT
9             ----------------------------------------------------------------------------------------------CAAATGAT
13,26,27      ----------------------------------------------------------------------------------------------CAAATGAT
18,19         ----------------------------------------------------------------------------------------------CAAATGAT
21            ----------------------------------------------------------------------------------------------CAAATGAT
20            ----------------------------------------------------------------------------------------------CAAACGAT
10            ----------------------------------------------------------------------------------------------CAAACGAT
16            ----------------------------------------------------------------------------------------------CAAACGAT
12            ----------------------------------------------------------------------------------------------CAAACGAT
```

Figure 3. Continued.

```
      gaps:        g
             401                                                    500

C. FLEXUOSA  TCACTTCATACATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTGAATACTGACAACAATGAA
R. PALUSTRIS TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
5,6,24,25    TTACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
7,8,23       TTACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
1,14,17      TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
2,3,4,11     TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
15           TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
9            TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
13,26,27     TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
18,19        TCACTT----GATAGTCTGATAAATCCTTGGTGAAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
21           TCACTT----GATAGTCTGATAAATCCTTGGTGAAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
20           TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
10           TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
16           TCACTT----CATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA
12           TCACTT----AATAGTCTGATAGATCCTTGGTGGAACTTATTAATCGGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATACTGACAACAATGAA

      gaps:                    abcdefg
             501         515

C. FLEXUOSA  ATTTATAGTAAGATG    1110111
R. PALUSTRIS ATTTATAGTAAGATG    1110100
5,6,24,25    ATTTATAGTAAGATG    1110110
7,8,23       ATTTATAGTAAGATG    1110110
1,14,17      ATTTATAGTAAGATG    1110010
2,3,4,11     ATTTATAGTAAGATG    0110010
15           ATTTATAGTAAGATG    0010010
9            ATTTATAGTAAGATG    0110010
13,26,27     ATTTATAGTAAGATG    0010010
18,19        ATTTATAGTAAGATG    1110010
21           ATTTATAGTAAGATG    1110010
20           ATTTATAGTAAGATG    1101010
10           ATTTATAGTAAGATG    1101010
16           ATTTATAGTAAGATG    1101010
12           ATTTATAGTAAGATG    1101010
```

Figure 3. Continued.

of *H. warburgii* and *C. zhejiangensis* formed one single cluster with no separation of *H. warburgii* versus *C. zhejiangensis*. This is in agreement with previous concepts combining both taxa, *C. zhejiangensis* and *H. warburgii*, in one single taxon. These congruent ITS and *trn*L intron findings demonstrate that both *Y. lichuanensis* and *Y. fumarioides* species complexes, treated as well-defined taxa according to the morphological revision of Al-Shehbaz et al. (1998), do not form monophyletic groups by molecular evidences.

However, some incongruencies among the two molecular data sets could be detected: (1) *Yinshania qianningensis* grouped either inside the *Hilliella/Cochleariella* clade (*trn*L intron data) or into *Yinshania* s. str. (ITS data); (2) *H. rupicola* (D. C. Zhang & J. Z. Shao) Y. H. Zhang also clustered into two different subgroups within *Hilliella/Cochleariella*.

TRIBAL RELATIONSHIPS

The alignment of ITS1–5.8SrDNA-ITS2 is 644bp in length (Fig. 4). Within the ITS1 region (bp 1–301) there are 143 variable nucleotide positions (including 62 autapomorphies). The ITS2 region (position 456–644) contains 85 variable nucleotide positions (including 57 autapomorphies). The 5.8S rDNA gene, located between both spacer regions (bp 302–455), contains 11 variable nucleotide positions (including 6 autapomorphies). Because of an ambiguous alignment, nucleotide positions 107–153 and 460–484 were removed from the original data matrix (Fig. 4), resulting in a final data matrix of 412 bp.

Fitch parsimony analysis resulted in one MPT with a length of 288 steps and a consistency index of 79.5% (66.5% if autapomorphies are excluded). The phylogenetic tree (Fig. 5) showed closer relationships of *Yinshania* to taxa from tribe Arabideae sensu Janchen (1942) (*Arabidopsis* (DC.) Heynh., *Barbarea* R. Br., and *Cardamine* L. were used to represent tribe Arabideae). However, *Cochlearia* (including species loosely affined to the *Yinshania* complex) and *Capsella rubella* were placed by Janchen in the tribe Lepidieae, where they had been put by Hayek (1911) and Schulz (1936). Our ITS data do not support this latter placement. A molecular analysis of *Arabidopsis*, *Arabis* L., and their relatives shows a close relationship of *Capsella rubella* to *Arabidopsis thaliana* (Koch et al., 1999b). Also demonstrated is the polyphyly of *Arabis* and *Arabidopsis*, indicating that tribal structures within Brassicaceae are highly artificial. The ITS phylogeny does suggest that Chinese *Yinshania* and related taxa are closer to *Cardamine* and *Barbarea* from tribe Arabideae than to genera like *Thlaspi* or *Cochlearia* s. str. of the tribe Lepidieae.

CYTOLOGY

Little is known about the cytology of *Yinshania*. Zhang (1995, and pers. comm.) counted $2n = 12$ for *Y. qianningensis* Y. H. Zhang [= *Y. acutangula* sensu Al-Shehbaz et al., 1998], *Y. henryi* (Oliv.) Y. H. Zhang, and *Y. furcatopilosa* (K. C. Kuan) Y. H. Zhang, $2n = 42$ for *Hilliella yixianensis* Y. H. Zhang, *H. paradoxa* (Hance) Y. H. Zhang & H. W. Li, and *H. changhuaensis* [= *Y. lichuanensis* sensu Al-Shehbaz et al., 1998], and $2n = 44$ for *H. shuangpaiensis* Z. Y. Li [= *Y. rupicola* sensu Al-Shehbaz et al., 1998]. These data correspond to the ITS-derived phylogeny, in which the diploid *Y. qianningensis*, *Y. henryi*, and *Y. furcatopilosa*, together with *Y. acutangula*, are separated from the polyploid *Hilliella*. Within polyploid *Hilliella*, the *Hilliella* taxa with $2n = 42$ are combined. *Hilliella shuangpaiensis* (represented in this study by acc. no. 4, Fig. 2) with $2n = 44$ did not group closely to the known $2n = 42$ taxa (represented in this study by acc. nos. 2, 11, and 15, Fig. 2). However, any conclusions based on cytology must be only preliminary. It remains possible that the *Hilliella/Cochleariella* group could also be represented by polyploid taxa with $2n = 42$ and 44. Base chromosome number for this hexaploid group is $x = 7$, instead of $x = 6$ as in the *Yinshania* group. A few taxa within the *Hilliella/Cochleariella* clade may be aneuploids ($2n = 44$) that derived from $2n = 42$.

DISTRIBUTION

Some interesting features emerge when topologies of the phylogenetic trees are compared to the geographic distribution of *Yinshania* s.l. Geographic distribution of ITS sequence types from taxa of the four main clades within *Hilliella/Cochleariella* do not follow their phylogenetic relationships (Fig. 7), and they are randomly mixed in Southeast China in the provinces of Guangdong, Jiangxi, Zhejang, Anhui, Hubei, and eastern Sichuan. However, they are separated geographically and phylogenetically from the *Yinshania* clade from Hunan, Xizang, and Sichuan. Taxa from the *Yinshania* clade extend the distribution to the southwest. A mixed distribution of DNA types also holds for the *trn*L data. No geographic structuring of plastome types could be observed among taxa from the *Hilliella/Cochleariella* clade (Fig. 8). Based on *trn*L intron data *Y. qianningensis* (acc. no. 1) from Sichuan integrates into the *Hilliella/Cochleariella* clade. Geographically,

```
               ITS1
               1                                                                                81
Arabidopsis    TCGATACCTGTCC-AAAACAGAACGACCCGCGAACCAA-AGATCACCA-CTCTCGGTGG-GCCGGTTTCTTAGCC-GATTC
Cardamine      TCGTATCCTGCCC-AAAAAAGACCGAACCGCGGACCAA-AGATCATCAACTCT-GGTGA-GCGGGTTTCATAACTTGAGAT
Cochlearia     TCGAAACCTGTTC-AAAACAGTACGACCCGAGAACAAC-TGATCATCA-CTCTCGGTAG-GCCGGTTTCTTAACA-GTTAT
Barbarea       TCGATACCTGTCC-AAAACAGAACGACCCGCGAACCAA-ATATCATCA-CTCTCGGTGG-GCCGGTTTCTTAGCT-GAGAT
Capsella       TCGATACCTGTCC-AAAACAGAACGACCCGAGAACCAA-AGATCACCA-CTCGCGGTAAAGCCGATTTCGTAACA-GATTT
Sinapis        TCGT-ATCTGG----AAACAGAACAACCCGAGAACAATGAAAACATCA-CTCTCGGTGG-GCCGGTTTCTTTGCT-GATTC
Brassica       TCGT-ACCCGGG---AAACAGAACGACCCGAGAACGTTGAAA-CATCA-CTCTCGGTGG-GCCGGTATCTTAGCT-GATTT
Yinshania      TCGATACCTGTCCAAAAACAGAACGACCCGCGAACCAA-AGATCATCA-CTCGCGGTAG-GCCGGTTTCTTAGCC-GATCC
Thlaspi        TCGTAACCTGTT-AAAAACAGAACGACCCGAGAACAAT-CGATCATCA-CTCTCGGTGG-GCCAGTTTCTTAAAT-GATCT


               82                        ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ       162
               CTTGCCC--GCCGGATCCGTGGTTTCGCGTATCGGCATGGTCGGGAGCTTTTATCTCGGTCTTGTC-----GTGCGCGTTG
               CGTGCCT--GCCGAATCCGTGGTTTCGCCGAACAATCCTTACCGGGAGCTTAATCTCTGTTTGGATT----GTGCGCGTTG
               CGTGCCT--ACCGATTCCGTGGTTTT-CATGTTTGGCTTCGTCAGTATTCATTACATGGCGAAGTC-----ATGCATGTTT
               CGTGTCTCTGCCGAATCCGTGGTTTCGTGAACAATCCTTACCGGGAGCTCTCTCTCTGTTTGGGTT-----GTGCGCGTAG
               CGTGCCT--GCCGAATCCGTGGTTTCCCGTACCTTCCCGGTCGAGAGTTTTCTCTCGGTCTGGTC------GTGCGCGTTG
               TGTGCCT--GCCGATTCCGTGGTT-ATGCGTTAAGTTCCCAGCCAGTACTTCAGTCTTGGTTGGGTC----TTGCGCATTG
               CGTGCCT--ACCGATTCCGTGGTT-ATGCGTTCGTCACCGGCCTAGNTTTCGGTTGGATT-----------GTACGCATAG
               CGTGCCT--GCCGAGTCCGTGGTTTCGCGTATTGTCCCGGGCGAGAGCTCAATCTCGGTCGTGTC------GTGCGCGTAG
               TGTGCCT--GCCGATTCCGTGGTTTCGCGTACGATTCTCATCAAGGTATATATATATATCTTGGTTTGATCATGCGTGTAG


               163                                                                             243
               CTTCCGGATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAAACGAACGGCTG--GCATTCGCCTCCCCGGAG
               CTTCCGGATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCAATTGAACAGACA--GCCTTCGCCTCCCCGGAG
               CCTCCGGATTTCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCTT--GCATTCGCCGCACCAGAG
               CTTCCGGATATCACAAAACCACGGCACGAAAAGTGTCAAGGAACATGCATATGAACAGCAA--GCCTTCGCCTCTCCAGAG
               CTTCCGGATATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACCGAACGGCTATAGCATTCGCTCCCCCGGAG
               CTTCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAGGTAGCCT--GCTTTCGCCAACCCGGAG
               CTTCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATTCAACTAAACAGCCT--NNTTTCGCCAACCCGGAG
               CTTCTGGATATCACCAAACCACGGCACGAAAAGTGTCAAGGAACATGCAACAGAACGGCCA--GCCTTCGCCTCCCCGGAT
               CTTCCGGTTATCACAAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGCCA--GCGTTTGCCTTCCCGGAG
```

Figure 4. Alignment of ITS1, 5.8S rDNA, and ITS2 regions from different crucifers. Characters excluded from data analysis are marked (Δ). For sequence accession data refer to the materials and methods section.

5.8SrDNA

```
244                                                                        324
ACGGAGTGTGG-GCGGATGCTGT-GCTGCGAACTGAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTGTGT-GCGGATCCTGC-GCTGCGATCTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ATGGTTTGTGT-GCGAATG-TCTCGATGC-ATTTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTGCGT-GCGTTTGGTGA-GCTGCGATCAAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTG-GTCGCGGATGCT-TAGCTGCGATATAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTTTGT-GCGGAAGCTGT-GCTCCAATGTAAAGTCTAAAACGACTCTCGGCAACGGATATCTTGGCTCTCGCATCG
ACGGTGTTTGT-TCGGAAGCAGT-GCTGCAATGTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCACATCG
ACGGTGTGAGT-GCGGATGTTGT-GCTGCGATCTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG
ACGGTGTTTGC-GTGAACGCTTT-GCTGCAATTTAAAGTCTAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCG

325                                                                        405
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC
ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC
ATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGC
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ATGAAGAAGCTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAAGCCAAGTTGCGC

                                                    ITS2
406                                                  ΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔΔ 486
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCCTCA-CCATCC-T-TTGCTGA-TGCGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-TC-TCATCC-TCTT-AGGACG-TGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCC-ATC-A-ATCC-TATA-AGGATTTT-GG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAACCGTCGTCCCTC--TCATTCTTCT-C-GGA-TATGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCC-CCCTCATCCTTCA--AGGATT-CGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCC-CC--CATCC-TCTCGAGGA-TATGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGTGTCACAAATCGTCGTCCN-CC--AATCC-TCTCGAGGA-TATCGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-CT-TCATCC-TTG-CG-GA-TACGGG
CCCAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTCACAAATCGTCGTCCC-C---CATCC-TCTTAAGGA-TACGGG
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Figure 4. Continued.

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487                                                                    567
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCTAAATCCGAGCC-AAGGACG-CCTGGAGCGTACCGACATG
ACGGAAGCTGGTCTCCCGTGTGTTACCGCACGCGGTTGGCCCAAATCCGAGC-TAAGGACG-CCGAGAGCGTCACGACATG
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ACGGAAGCTGGTCTCCCGTGTTTTACCGAATGCGGT-GGCCAAAATCTGAGC-TAAGGACG-CCAGGAGTGTCTCGACATG

568                                                                    644
CGGTGGTGAACTTGATCCATTA-CA-TTTTATCGGTCGCTCTTGTCCGGAAGCTGTAGATGACCCAAAGTCCATATA
CGGTGGTGAACTAAAGCCTGT-TCG-TATCGTCGGTCGTTCTTGTCTATAAGCTCTCGATGACCCAAAATCCTCAAA
CGGTGGTGAAAT-AAGCCTCT-TCA-TAACGTCGTGCGCTCTTGTCCAAAAGCTCCCGATGACCCAATGTCTTCTAT
CGGTGGTGAACTAAAGCCCCT-TCG-TATTGTCGGTCGTTCTTGTCCGGAAACTCTCGATGACCCAAAGTCTTTAAA
CGGTGGTGAACTCGTTCAACTCTCCCTATCGTCGGTCGCTCTTGTCCGGAAGCTCTAGATGACCCAAAGTCTTCAAT
CGGTGGTGAATTGTAACCTCG-TCA-TATTGTCGGTCGTTCCGGTTCAAAAGCTCTTGATGACCCAAAGTCCTCAAC
CGGTGGTGAATTCAATTCTCG-TCA-AATCGTCAGTCGTTTCGGTCCGAAAGCTCTTGATGACCCAAAGTCCTCAAC
CGGTGGTGTGTGCAAGCCTCTATAA-TATCGTCGGCCGCTCTTGTCCG-AAGCTCTA-AT-ACCCAAAGTT-TCAAG
CGGTGGTGAATTCAAGCCTCTTTAG-T-TTGTCGGCCGCTCTTGTCTGGAAGCTCTTGATGACCCAAAGTCCTCAAC
```

Figure 4. Continued.

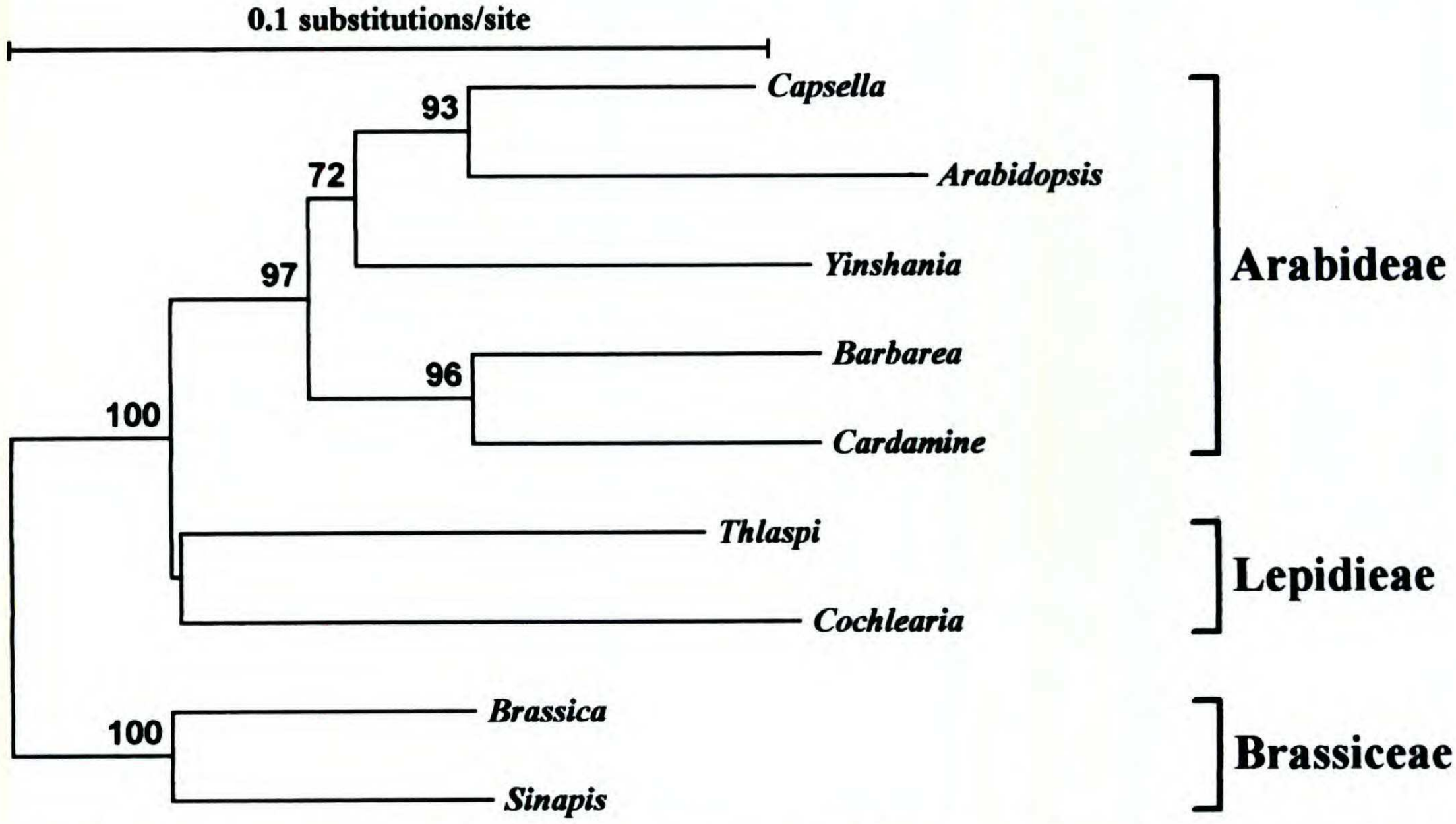

Figure 5. Phylogenetic relationships of cruciferous taxa from different tribes. Genetic distances were calculated using the program TREECON (van de Peer & de Wachter, 1994) under the Kimura model (Kimura, 1980). Gaps were not taken into account, and the neighbor joining algorithm was used to calculate genetic distances. The robustness of the tree was tested by 1000 bootstrap replicates.

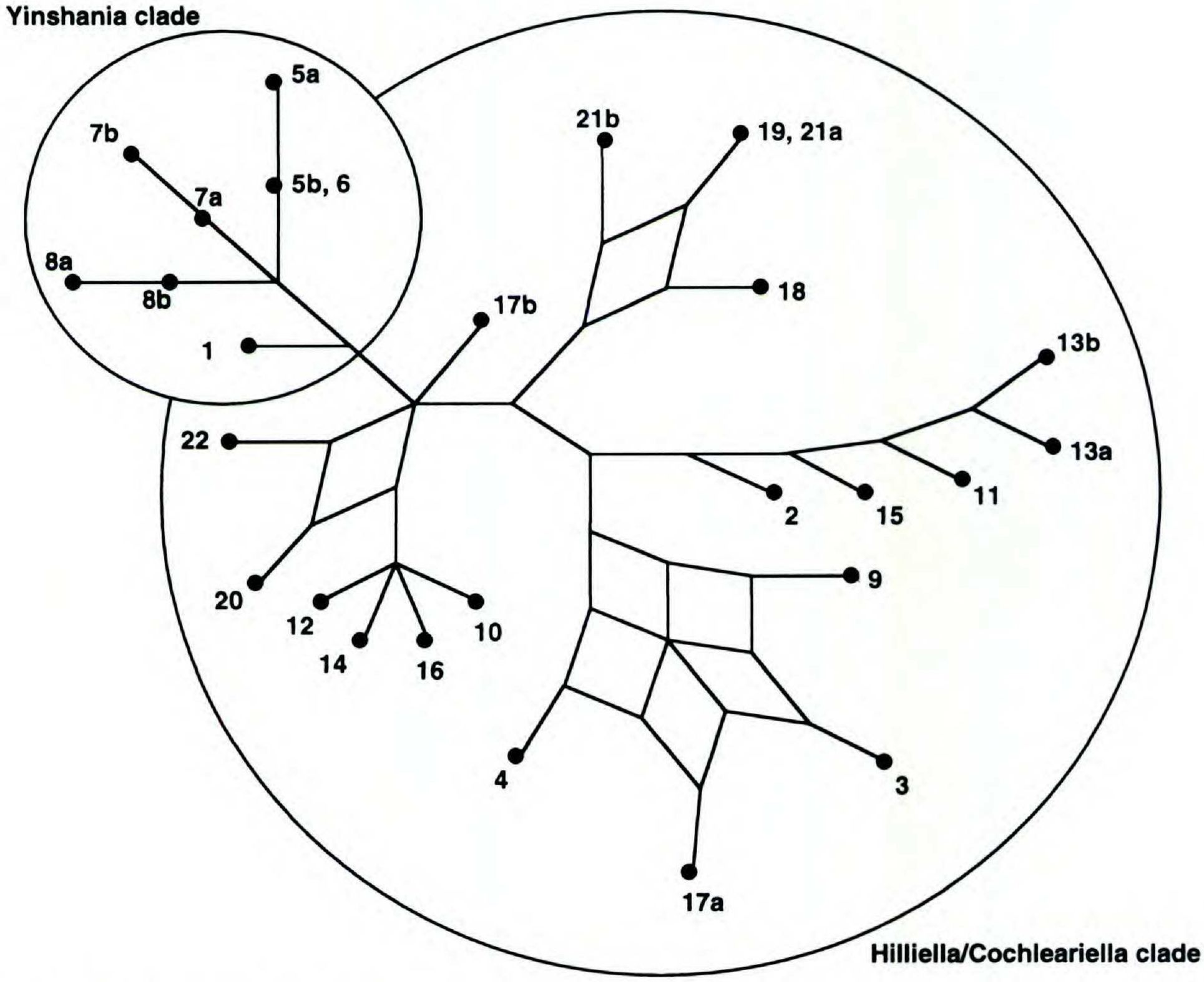

Figure 6. Split Decomposition of the ITS sequence data set analyzing the ingroup taxa. The DRAW-EQUAL-EDGES option was used to draw the network, and thus distances are not drawn to scale.

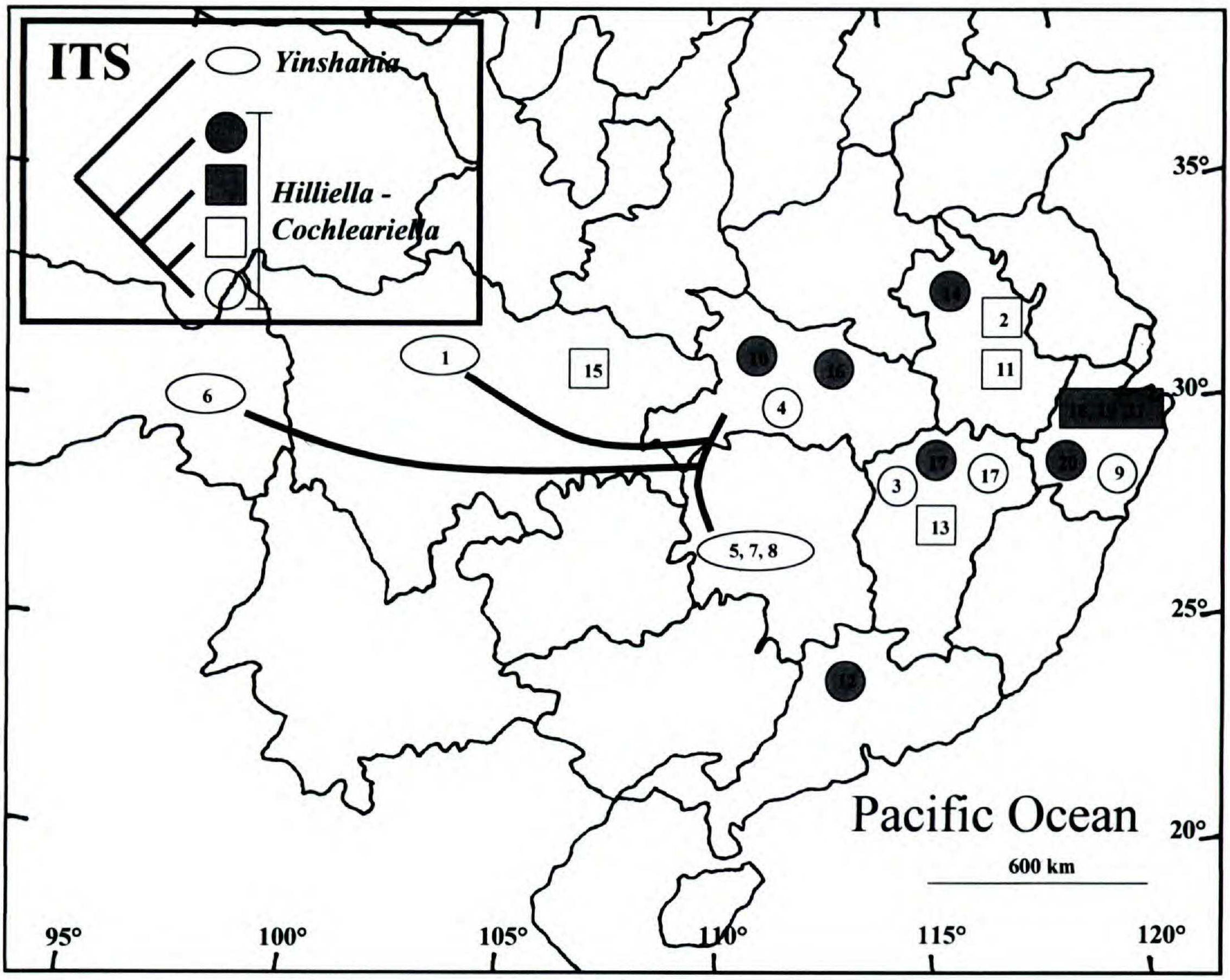

Figure 7. Distribution of ITS types among *Yinshania* and *Hilliella/Cochleariella* accessions under study in China. Taxon enumeration follows Table 1. The *Hilliella-Cochleariella* clade is separated into four ITS types indicated by circles and boxes (gray or white) well suppported by high bootstrap values in Figure 2. Accessions marked by open circles and boxes are two well-separated subgroups, which are combined to a single group in Figure 8 (plastid *trn*L data). Phylogenetic relationships are shown schematically in the upper left box and follow Figure 2. For the *Yinshania* clade phylogenetic relationships are shown to demonstrate the position of *Yinshania qianningensis* [*Y. acutangula* sensu Al-Shehbaz] (acc. no. 1) (refer to Fig. 2).

this accession lies close to a similar plastome type (acc. no. 15) from the *Hilliella/Cochleariella* clade (Fig. 8). Upon comparison of the ITS and *trn*L intron-derived phylogenies, taxa that showed different positions in the phylogenetic analysis (e.g., *H. rupicola*, acc. no. 14; *H. fumarioides*, acc. no. 17: one additional ITS sequence copy) were located at the center of the *Hilliella/Cochleariella* distributional area. We interpret these results as a first biogeographical documentation of reticulation within polyploids. *Hilliella fumarioides* (acc. no. 17) possessed a *trn*L intron type similar to samples from surrounding areas. The two ITS types found in that particular individual were also present in adjacent regions and from different species. From *Hilliella rupicola* (acc. no. 14) we could isolate an ITS DNA type, which is also present in adjacent regions in accession numbers 10, 16, 17, and 20. *trn*L intron DNA type from accession number 14 also corresponds to *trn*L intron DNA types from 2, 3, 11, 13, 17, and 20.

Morphological Variation and Taxonomic Considerations

As in numerous other cases in the Brassicaceae, morphological differentiation among and within so-called genera and taxa of the *Yinshania* s.l. complex does not provide ample characters to draw sharp and uncontroversial generic boundaries. Flower morphology in the entire complex is of no predictive diagnostic value, even for the separation of species (Al-Shehbaz et al., 1998). Only leaves and fruits offer characters useful for the separation of species, and all taxa appear to be not well defined. As shown by Al-Shehbaz et al. (1998), seed sculpture and number per locule, cotyledonary position, development of the fruit septum, type (if any)

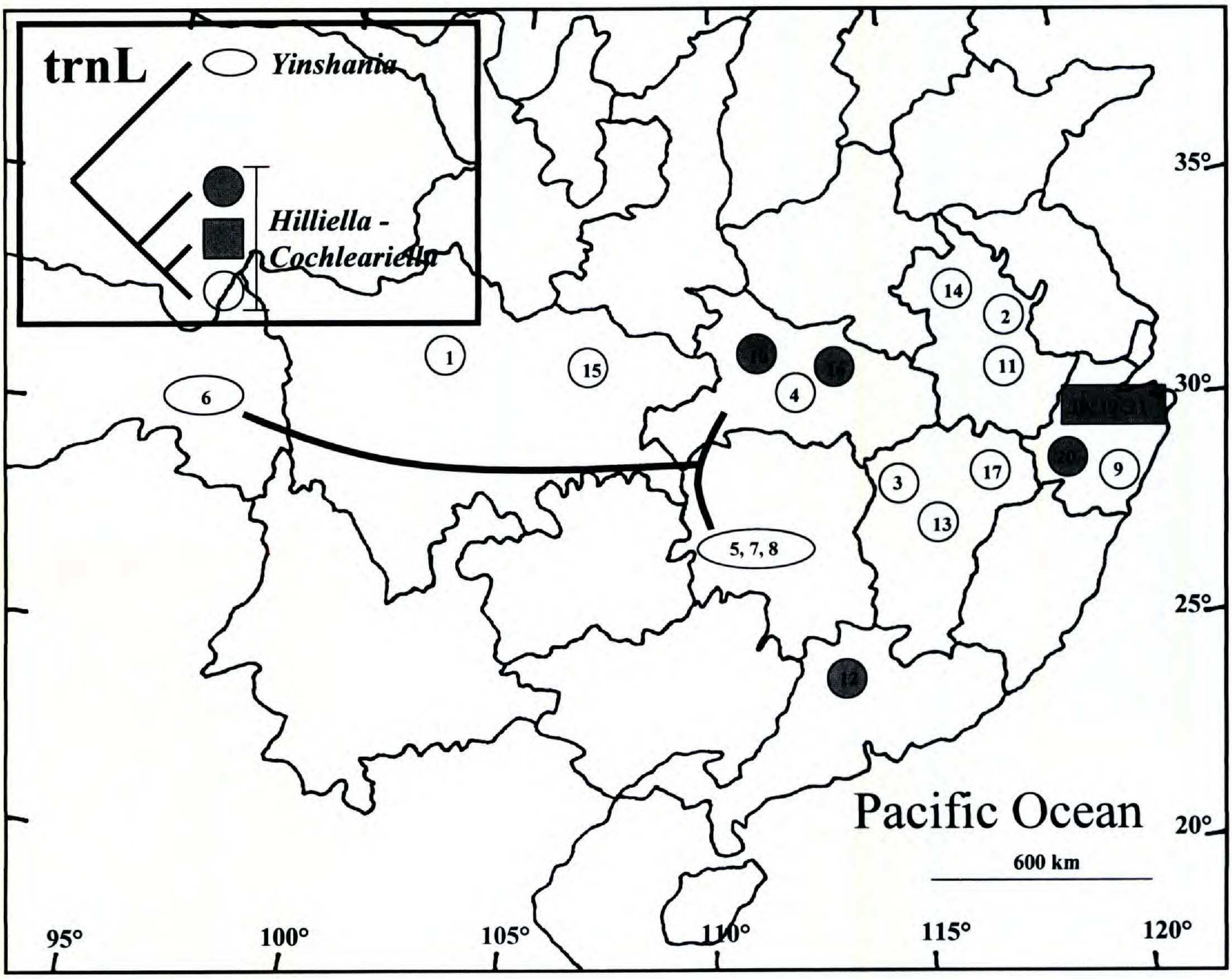

Figure 8. Distribution of *trn*L intron types among *Yinshania* and *Hilliella/Cochleariella* accessions under study in China. Taxon enumeration follows Table 1. The *Hilliella-Cochleariella* clade is separated into three plastid *trn*L types indicated by circles and boxes (gray or white) well suppported by high bootstrap values in Figure 2. Phylogenetic relationships are shown schematically in the upper left box and follow Figure 2. For the *Yinshania* clade phylogenetic relationships are shown to demonstrate the position of *Yinshania qianningensis* [*Y. acutangula* sensu Al-Shehbaz] (acc. no. 1) (refer to Fig. 2).

of fruit compression, fruit shape, development of papillae on the fruit valve, and trichome type, all are unreliable in dividing the complex into the genera *Yinshania, Hilliella,* and *Cochleariella.* In fact, no single character or set of characters can be relied upon to subdivide the complex into generic or infrageneric taxa. The recognition of a single genus is, therefore, taxonomically expedient, as there is no single character setting one clade apart from another. Even pustules on the testa surface of the valves, which were assumed to be a good character to separate *Yinshania* (pustules present) from *Hilliella* (Zhang & Xu, 1990), appear not to be congruent, failing to provide a good argument to split *Yinshania* into several genera (Al-Shehbaz et al., 1998).

Morphological Cluster Analysis

A cluster analysis of morphological and ecological characters has been performed by Zhang and Xu (1990). This analysis considered distribution, altitude, and habit, but also morphological characters describing hairs, inflorescences, flower details, silicles, seeds, and cotyledons. They concluded that minute pustules on the testa surface of the valves were a good character to separate *Yinshania* from *Hilliella.* Comparison of our molecular phylogenies with this cluster analysis based on 32 morphological and 3 ecological characters demonstrated the lack of congruency between morphological and molecular evolution. This cluster analysis separated *Yinshania* sensu Ma and Zhao (1979) from *Hilliella* sensu Zhang and Li (Zhang, 1986) (a similar resolution to the ITS data). Taxa such as *H. paradoxa, H. lichuanensis,* and *H. changhuanensis* are closely related to each other (as confirmed by molecular data by acc. nos. 11, 13, and 15 herein). However, this morphological cluster analysis also grouped *H. guangdongensis* (acc. no. 12 herein)

into this group (*Y. lichuanensis* clade, Fig. 2). For both ITS and *trn*L intron sequence, *H. guangdongensis* is very divergent from others grouping with the *Y. lichuanensis* clade (Fig. 2). *Hilliella alatipes* var. *micrantha* and *H. sinuata* are separated with both molecular markers (Fig. 2), but are combined as a well-supported group in morphological cluster analysis (Zhang & Xu, 1990). In summary, within *Hilliella* there is little morphological agreement with our molecular data: (1) *H. paradoxa, H. changhuaensis,* and *H. lichuanensis* are closely related to each other (as *Y. lichuanensis* clade, Fig. 2); (2) *H. hunanensis* and *H. rupicola* grouped together on the ITS tree (but not based on *trn*L data, Fig. 2). No further correlation could be observed. None of the three data sets (ITS, *trn*L intron, morphology) is powerful enough to elucidate phylogenetic signal for the whole species complex. However, significant correlations could be observed when cpDNA- and nrDNA-derived phylogenies were compared, dividing the *Hilliella/Cochleariella* clade into several subgroups (Fig. 2) clearly separating *Yinshania* relatives from *Hilliella/Cochleariella* with the sole exception of *Y. qianningensis.*

Hybridization, Introgression, Chloroplast Capture, and Concerted Evolution

The phylogeny based on plastid *trn*L intron sequence data reflects the maternal lineages because plastids are inherited maternally in most angiosperms, including the Brassicaceae (Harris & Ingram, 1991; Reboud & Zeyl, 1994). Introgression of a chloroplast type characteristic for the *Hilliella/Cochleariella* clade into *Yinshania qianningensis* demonstrates possible gene flow between both groups. Because of the highly polyploid genomes of *H. yixianensis, H. paradoxa, H. changhuaensis,* and *H. shuangpaiensis* (represented by acc. nos. 2, 15, 11, and 4, respectively, in Fig. 2) with multiple rDNA loci, and the assumed hybridization within the *Hilliella/Cochleariella* clade and even with the *Yinshania* clade, there is a high probability of concerted ITS sequence evolution. In principle, there are three different ways that two different ITS copies evolve within a single individual: (1) unidirectional concerted evolution leads to the loss of one copy and fixation of the second (detected in *H. rupicola* acc. no. 14, herein; and in *Gossypium,* Wendel et al., 1995a); (2) both ITS copies are still present, which might be mostly the case in young hybridogenous taxa (detected in *H. fumarioides* acc. no. 17, and *C. zhejiangensis* acc. no. 21, Fig. 2; in *Krigia,* Kim & Jansen, 1994; in *Arabidopsis,* O'Kane et al., 1996); and (3) concerted evolution leads to a new ITS type that represents a mixture of the two original ITS sequences (in *Gossypium,* Wendel et al., 1995b; in *Microseris,* van Houten et al., 1993; in *Microthlaspi,* Mummenhoff et al., 1997). The third type of concerted evolution might have happened in *H. sinuata* (acc. no. 3). This accession showed a plastome type more similar to *H. shuangpaiensis* (acc. no. 4). However, ITS sequence types from putative parental ITS sequence types from *H. sinuata* (acc. no. 9) and *H. shuangpaiensis* (acc. no. 4) exhibit some additive features found in *H. sinuata* (acc. no. 3). Comparing these three sequences, there are 39 variable nucleotide positions (21 in ITS1 region, and 18 in ITS2 region). Within the ITS1 region, 17 out of 21 variable nucleotide positions (81%) are identical among the two *H. sinuata* accessions (nos. 3, 9); *H. shuangpaiensis* shared only three mutations with *H. sinuata* (acc. no. 9) and one mutation with *H. sinuata* (acc. no. 3). Within the ITS2 region both *H. sinuata* accessions have only one nucleotide position out of 18 variable nucleotide sites invariant, but *H. shuangpaiensis* (acc. no. 4) shared 15 positions (83%) with *H. sinuata* (acc. no. 3) and two positions with *H. sinuata* (acc. no. 9). These findings indicate that ITS type of *H. sinuata* (acc. no. 3) consists of a mixture of ITS types from relatives of *H. shuangpaiensis* (mostly ITS2 region) and *H. sinuata* (ITS1 region). The overall sequence divergence between *H. shuangpaiensis* (acc. no. 4) and *H. sinuata* (acc. no. 9) is 7.8%.

Concerted evolution of ITS DNA loci has been shown several times to occur in the Brassicaceae (O'Kane et al., 1996; Mummenhoff et al., 1997; Koch et al., 1998b; Franzke et al., 1998) and other families (Wendel et al., 1995a, b; Buckler et al., 1997). Sequence divergence values of ITS types from putative parents, giving rise to hybrids in which concerted evolution has been observed, ranged from 3.1% (*Microthlaspi natolicum* vs. *M. perfoliatum,* Mummenhoff et al., 1997), 5.0% (*Cardamine amara* vs. *C. rivularis* auct., Franzke et al., 1998), to 6% (*Arabidopsis thaliana* vs. *A. arenosa,* O'Kane et al., 1996) comparable to a value of 7.8% found among *H. shuangpaiensis* versus *H. sinuata.*

We conducted a split decomposition analysis to visualize conflicting phylogenetic signal indicating concerted evolution in groups among the *Hilliella/Cochleariella* clade (Fig. 6). This analysis clearly indicates hybridization with subsequent concerted evolution of ITS regions in *H. sinuata* (acc. no. 3). Concerted evolution of ITS sequences greatly influences any interpretation of the ITS phylogeny. Since diploid members of the Brassicaceae such as *Arabidopsis thaliana* typically show 2 NOR loci,

one could assume that in hexaploid *Hilliella/Cochleariella* taxa at least 6 major NOR loci are present. Therefore, sequencing of two individual ITS clones is not a sufficient survey to find all putative ITS types from a single individual. This undersampling leads to an underestimation of ITS type variation as well as the degree of hybridization and concerted evolution.

We found evidence suggesting concerted evolution and described three examples (*H. fumarioides* acc. no. 17, *H. rupicola* acc. no. 14, *H. sinuata* acc. no. 3) of possible hybridization and subsequent concerted evolution of ITS sequence. The overall amount of sequence divergence between taxa in this study demonstrates a relatively high age for the different lineages. In fact, sequence divergence values are much higher when compared to those obtained in infrageneric studies of closely related species of other Brassicaceae (e.g., *Cochlearia* s. str. < 1.75%, Koch et al. (1999a); *Noccaea* < 4.5%, Mummenhoff et al. (1997); *Cardamine* < 4.5%, Franzke et al. (1998)). Within the *Yinshania* clade, sequence distance values range up to 6%, but within the *Hilliella/Cochleariella* clade they range even higher, up to 27%.

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